Short-term versus long-term effects of nitrogen addition and warming on ecosystem N dynamics in a grass-dominated temperate old field

Benjamin F. A. Souriol, Western University

Supervisor: Dr. Hugh Henry, The University of Western Ontario

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology
© Benjamin F. A. Souriol 2023

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Biochemistry Commons, Biogeochemistry Commons, Biology Commons, and the Terrestrial and Aquatic Ecology Commons

Recommended Citation
https://ir.lib.uwo.ca/etd/9713

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlswadmin@uwo.ca.
Abstract

Atmospheric nitrogen (N) deposition and climate warming are both anticipated to influence the ecosystem N dynamics of northern temperate ecosystems substantially over the next century. Temporal scale can play an important role in determining the extent of treatment effects on N dynamics, and it is unclear to what extent the results of short-term studies can be extrapolated to responses over longer time scales. I compared the short-term (3 year old) versus long-term (16 years old) effects of N addition and warming on net N mineralization, N leaching, and N retention in a grass-dominated old field. There was a significant interaction between plot age and N addition over the growing season. However, there were no significant interactions between either of the treatments and plot age for $^{15}$N retention. This result might be attributed to the importance of plant community composition shifts in response to global change treatments during field studies.

Keywords: Nitrogen, warming, atmospheric deposition, net mineralization, leaching, retention, $^{15}$N, short-term versus long-term, interannual variation, soil, temperate, grassland.
Summary for Lay Audience

Nitrogen (N) is a key element in many ecosystems, given that the availability of N in forms that can be taken up by plants often limits plant growth and influences species composition. Since the industrial revolution, the quantity of biologically available N resulting from human actions has increased by an order of a magnitude. A large majority of this N is applied directly to agricultural fields in excess amounts. The excess N that does not become taken up by plants or soil microorganisms can pollute the soil, water, and atmosphere. Likewise, the burning of fossil fuels can contribute to atmospheric N pollution. Atmospheric N pollution can be carried long distances, and subsequently be deposited back onto the landscape in precipitation or via dust, potentially disrupting natural ecosystems. Climate warming is another important factor that can alter N in ecosystems. In field experiments with N addition and warming treatments, time scale can play an important role in determining the extent of treatment effects on N dynamics. I compared the short-term (3 year) versus long-term effects (16 year) of N addition and warming on N availability and N retention in a grass-dominated field. I examined the response of N mineralization, whereby soil microorganisms break down decaying organic matter to obtain energy and, in the process, can release N back to the soil. I also examined N losses via leaching (losses of N in the water draining through the soil), and I added N labelled with $^{15}$N, the heavy isotope of N, to trace the retention of N added to the soil. For N mineralization, plot age influenced the warming and N addition effects to some extent, but plot age did not influence their effects on $^{15}$N retention. The lack of treatment effects on the plant composition of the plots after 16 years may explain why plot age was not an important factor in influencing the effects of the treatments on $^{15}$N retention.
Co-Authorship Statement

Dr. Hugh Henry will be a co-author on any manuscripts published from the data chapters of this thesis. Dr. Henry has offered me considerable guidance during the research, design, and implementation of the experimental design, as well as during analysis of the data and interpretation of the results.
Dedication

I dedicate this thesis to my two companions; Kara and Little Bones. I also dedicate this thesis to my wife Katrina and my mother Donna Lee; I never would have made it here without their help, love, and support.
Acknowledgements

Firstly, I thank my supervisor, Dr. Hugh Henry. Hugh has been an amazing mentor and friend to me throughout my MSc project, with invaluable knowledge and insight into my project and education, putting up with my shenanigans, and supporting me every step of the way. I also thank my family here in London; Julie & Dan, as well as everyone who encourages me along the way and all my friends who were with me during this journey. I thank my advisory committee, Dr. Zoë Lindo and Dr. Greg Thorn, for all their advice and suggestions. I would also like to thank all my lab mates and everyone who I have met here at The University of Western Ontario who make this institution a great place to learn and grow.
# Table of contents

Certificate of Examination ................................................................. ii  
Abstract and Keywords ........................................................................ iii  
Summary for Lay Audience ..................................................................... iv  
Co-authorship Statement ...................................................................... v  
Dedication ............................................................................................. vi  
Acknowledgements ............................................................................... vii  
Table of Contents ................................................................................ viii  
List of Tables ....................................................................................... x  
List of Figures ....................................................................................... xi  
List of Abbreviations ........................................................................... xii

## 1. General Introduction

1.1 Scientific Rationale ................................................................. 1  
1.2 Objectives and Hypotheses .................................................. 17  
1.3 Thesis Organization .............................................................. 18  
1.4 References ............................................................................... 19

## 2. Short- versus long-term effects of nitrogen addition and warming on soil nitrogen mineralization and leaching

2.1 Introduction ............................................................................... 39  
2.2 Methods ................................................................................... 42  
   2.2.1 Site description ...................................................................... 42  
   2.2.2 Warming and N addition treatments .................................... 42  
   2.2.3 Net N mineralization and leaching ....................................... 46  
   2.2.4 Statistical analyses ............................................................. 52  
2.3 Results .................................................................................... 52  
   2.3.1 Net N mineralization and leaching from spring to peak growing season ....52  
   2.3.2 Net N mineralization and leaching from peak biomass to first snowfall ....56  
   2.3.3 Net N mineralization and leaching over winter .................... 60  
2.4 Discussion ................................................................................ 63  
2.5 Conclusion ............................................................................... 66  
2.6 References ............................................................................... 67

## 3. Short- versus long-term effects of nitrogen addition and warming on ecosystem nitrogen retention

3.1 Introduction ............................................................................... 75  
3.2 Methods ................................................................................... 78  
   3.2.1 Field site description ........................................................ 78  
   3.2.2 Warming and N addition treatments .................................... 78  
   3.2.3 $^{15}$N tracer addition and analyses ...................................... 84  
   3.2.4 Statistical analyses ............................................................. 85  
3.3 Results .................................................................................... 85  
   3.3.1 Recovery of the $^{15}$N label in Bromus inermis aboveground biomass ........85  
   3.3.2 Recovery of the $^{15}$N label in Poa pratensis aboveground biomass .......86
3.3.3 Recovery of the ¹⁵N label in soil .................................................................93
3.4 Discussion ........................................................................................................93
3.5 Conclusion .........................................................................................................97
3.6 References.........................................................................................................97

4. General Discussion & Conclusions
4.1 Research findings.............................................................................................110
4.2 Research limitations .........................................................................................112
4.3 Directions for future research ..........................................................................113
4.4 References.........................................................................................................114

Curriculum vitae ....................................................................................................124
List of Tables

Table 2.1 P values for three two-way ANOVAs testing for effects of 1) plot age, N addition and their interaction, 2) plot age, warming, and their interaction, and 3) N addition, warming, and their interaction on change in soil extractable inorganic N, resin extractable N, and total N mineralization ..........................................................55

Table 2.2 Initial, final, and resin extractable ammonium and nitrate over the growing season, peak growing season to first snowfall, and over winter...............................57

Table 3.1 P values for three two-way ANOVAs testing for effects of 1) plot age, N addition and their interaction, 2) plot age, warming, and their interaction, and 3) N addition, warming, and their interaction on % recovery of the $^{15}$N isotope tracer in aboveground Bromus inermis biomass, aboveground Poa pratensis biomass, and bulk soil samples .................................................................91

Table 3.2 Percent N of Bromus inermis, Poa pratensis, and bulk soil samples..............92
List of Figures

Figure 1.1 Inputs, outputs, and internal cycling of nitrogen in an ecosystem..............10

Figure 2.1 Plot design showing a representative block for Chapter 2’s experiment........44

Figure 2.2 Design of the in situ soil cores.................................................................47

Figure 2.3 A) daily mean ambient air temperature, B) daily precipitation, and C) snow
cover over the duration of the soil N mineralization and leaching measurements.
Sampling periods for the in situ soil cores are also indicated....................................50

Figure 2.4 A) Total N mineralization/immobilization, B) resin extractable N, and C)
changes in soil extractable N from spring to peak growing season ................................53

Figure 2.5 A) Total N mineralization/immobilization, B) resin extractable N, and C)
changes in soil extractable N from peak growing season to first snowfall ....................58

Figure 2.6 A) Total N mineralization/immobilization, B) resin extractable N, and C)
changes in soil extractable N over winter........................................................................61

Figure 3.1 Plot design showing a representative block for Chapter 3’s experiment.......80

Figure 3.2 A) daily mean ambient air temperature, B) daily precipitation, and C) snow
cover over the duration of the \(^{15}\)N study. Dates for timing of the \(^{15}\)N isotope addition and
subsequent collecting are indicated................................................................................82

Figure 3.3 Mean aboveground biomass of A) Bromus inermis and B) Poa pratensis
samples at the end of the growing season...............................................................87

Figure 3.4 Mean % recovery of the \(^{15}\)N label in A) Bromus inermis, B) Poa pratensis,
and C) bulk soil samples............................................................................................89
List of abbreviations

N – Nitrogen

$^{15}$N – ‘heavy’ stable isotope of nitrogen, molecular weight of 15

C – Carbon

NH$_4$NO$_3$ – ammonium nitrate

$^{15}$NH$_4$$^{15}$NO$_3$ – ammonium nitrate $^{15}$N tracer

PVC – polyvinyl chloride

$\delta$$^{15}$N – sample $^{15}$N enrichment relative to the natural abundance standard
Chapter 1

General Introduction

1.1 Scientific Rationale

Nitrogen as a limiting nutrient for plant growth

The nitrogen (N) cycle is unique in that N is in great supply on our planet but it primarily exists in a form that is unavailable for uptake by most organisms (White 1993). Nitrogen is the most abundant element found in Earth’s atmosphere, comprising 78% of the atmosphere’s volume (Galloway et al. 2004), yet 99.95% of this atmospheric N pool is in the form of the inert nitrogen gas N₂, which is strongly bound together by a covalent triple bond (Galloway et al. 1994; Greenwood & Earnshaw 1984). Very few biological organisms are capable of breaking this bond, resulting in a relatively small pool of N that is available to be taken up by plants and animals (Delwiche 1970; Vitousek et al. 1997). However, N is in high demand by plants and animals due to its requirement in building nucleic acids and proteins (Chen et al. 2022), and it also is an essential component of chlorophyll, the primary pigment required in the light capturing process of photosynthesis (Senge et al. 2014). Inorganic forms of N, such as ammonia (NH₃), ammonium (NH₄⁺), nitrite (NO₂⁻), and nitrate (NO₃⁻), are referred to as reactive N because they are available for uptake by plants and microbes (Sokolov et al. 1996). Nevertheless, a large proportion of the N contained in ecosystems is in organic forms in plant, animal and microbial biomass, and in soil; this organic N is considered immobilized because it cannot be taken up by most plants, with the exception of simple organic molecules such as urea or amino
acids (Mulvaney et al. 2001; Svennerstam & Jamtgard 2022). This mismatch between N supply in available forms and N demand results in N being the nutrient that limits primary productivity in the majority of terrestrial ecosystems (Glough 2011), particularly in temperate regions and at high latitudes (Fay et al. 2015).

*Nitrogen cycling in terrestrial ecosystems*

The quantity of N that undergoes internal transformations and cycling inside an ecosystem greatly outweighs inputs and outputs of N, leading to the N cycle being considered a relatively closed system (Bernhard 2010). Approximately only ~2% of the N that exists in soils is in the biologically active form while ~98% is in organic form, bound in litter or particulate matter (Angus 2001; Schlesinger 2009), therefore the key transformation of N in an ecosystem is the conversion of plant-inaccessible organic N (e.g. proteins and peptides) to mineral N in a process termed mineralization. The first step of this process is referred to as ammonia-oxidization or ammonification; heterotrophic soil microorganisms, normally fungi and prokaryotes, break down organic matter to utilize the carbon skeleton for their energetic demands (Xing et al. 2019). Because these decomposers have a high N use efficiency, they require less N per unit biomass produced than their substrate (high C/N ratio) and therefore they incorporate the carbon into their tissue and release a net positive amount of N back to the environment, usually in the form of ammonia (Konneke et al. 2005). Ammonia, once available in the soil, can react with water to form ammonium or undergo nitrification to form nitrate in two distinct steps: first ammonia is converted to nitrite by ammonia-oxidizer microbes (*Nitrosomonas, Nitrospira, Nitrosococcus*, and *Nitrosopumilus*) (Lehtovirta-Morley 2018), then nitrite is
oxidized further to nitrate by a nitrite-oxidizing bacteria (*Nitrospira, Nitrobacter, Nitrococcus, Nitrospina*) (Daims et al. 2016). At any step of these pathways, the various forms of reactive N produced may be taken up by microbes and plants and immobilized. Soil N also can become incorporated into soil aggregates or occlusions, protecting N from uptake by plants. Moreover, inorganic N molecules with a positive electrical charge (such as ammonium and the amine group on amino acids) can become loosely bound to the negative charge found on the surface of clay particles or organic matter, with the number of charges (i.e. monovalent vs. polyvalent) and hydrated radius being the two factors that determine the strength of this interaction, through a phenomenon known as cation exchange capacity (Nieder et al. 2011; Zhu et al. 2019). Similarly, negatively charged molecules can be bound to positively charge soil particles through anion exchange capacity; however, this mechanism is not as prevalent and is mostly a factor in tropical ecosystems due to the increase of weathered minerals and decreased pH (Wang et al. 2013). Mineral N that does not become incorporated into biomass or become a part of soil aggregates is susceptible to losses from the ecosystem.

**Nitrogen inputs to terrestrial ecosystems**

The major pathway for the input of reactive forms of N into an ecosystem is through biological fixation, which is carried out by both free-living bacteria and bacteria living in symbiosis with specific species of plants (Vitousek et al. 2002). *Rhizobium* and *Frankia* are diazotrophic bacteria that fix N from the atmosphere and convert it into biologically active forms (Liu et al. 2011). *Rhizobium* bacteria are housed inside nodules found on the roots of legume plants, whereas *Frankia* live in symbiosis with actinorhizal
plants; these root nodules protect the bacteria from external environments, predation, and enzymes that reduce the efficiency of their N fixation (Vitousek et al. 2002). *Nostoc* and *Azotobacter* are free-living cyanobacteria that have the potential for both N fixation and photosynthesis, allowing them to produce their own energy as well as convert nitrogen gas into reactive forms that can be later absorbed by plants (Tsygankov 2007). There is also a minor pathway that allows some reactive N to enter ecosystems during storm events. Specifically, the energy associated with lightning strikes has the potential to split the covalent bond in nitrogen gas. Once free, these N atoms bond with oxygen gas (O₂) in the atmosphere and create nitrogen oxides (NOₓ) that can be later dissolved in precipitation and enter ecosystems as nitrate (Hill et al. 1980). Similarly, during the eruption of volcanoes, large amounts of gaseous N is produced and may enter the atmosphere.

*Nitrogen outputs from terrestrial ecosystems*

The two main pathways of N loss from terrestrial ecosystems are through gaseous losses to the atmosphere and hydrological processes. Denitrification, which is the dominant pathway of gaseous N loss from ecosystems, is an anaerobic process performed by denitrifying bacteria (e.g. *Bacillus*, *Paracoccus*, and *Pseudomonas*), in which nitrate is converted into nitrogen gas; nitric oxide (NO) and nitrous oxide (N₂O) are intermediate gases in this pathway, with the latter being a potent greenhouse gas that contributes to climate warming (Risgaard-Petersen et al. 2006; Ward et al. 2009). Anaerobic ammonia-oxidization (or anammox) is another pathway that produces nitrogen gas from ammonium, while using nitrite as an electron acceptor under anoxic conditions; this
process is carried out by prokaryotes in the Planctomycetota phylum (Strous et al. 1999). Ammonia gas also may be lost from the soil to the atmosphere through a process known as ammonia volatilization; any of the aforementioned processes that convert N into ammonia therefore increase the risk of this form of gaseous N loss (Cameron et al. 2013). Nitrogen that is not immobilized in biomass or occluded in soil matrices can also become dissolved in water and lost by leaching through the soil profile into ground water or lost laterally as water run-off. While both labile organic N that is dissolved in water (DON) and mineral N dissolved in water (DIN) are susceptible to losses through these hydrological pathways, nitrate is the most mobile form of N in the soil, and it therefore poses the greatest risk of hydrologic N loss from ecosystems (Hanrahan et al. 2019).

Disturbance also can promote N losses from ecosystems. Fire can cause gaseous losses of N through the combustion of organic matter, while floods, landslides, and windthrow may be physical pathways for the loss of both inorganic and organic N (Sever 2020). Herbivory also can affect N cycling within an ecosystem, transforming the pool of organic N from plant to animal. Animals may alter the flux of N into or out of a system but the migration of herbivorous animals into or out of ecosystems is usually considered to be a relatively small flux of N (Habeck & Meehan 2008). However, in managed ecosystems, biomass removal during harvest of crops in agricultural fields or timber from forests and the subsequent transportation for human consumption is another example of this migrational flow (Evans & Belnap 1999; Vitousek & Matson 1985).
Atmospheric nitrogen deposition

Since the first industrial revolution (1850 – present), humans have augmented the pathways for the input of reactive N into ecosystems; 60% of atmospheric N deposition now originates from food production (agricultural activity) and energy production (the burning of fossil fuels for transport and industry) (Gruber & Galloway 2008). Anthropogenic sources of biologically active forms of N have increased by an order of a magnitude, from ~15 Tg N in 1860 to ~156 Tg N in 1995, and then 187 Tg N in 2005 (Galloway et al. 2008). The main culprit for this increase is the advent of the Haber-Bosch reaction, which has allowed the production of ammonia using nitrogen gas and hydrogen gas (H₂) (Janovic & Jukic 2018). Most of the products of this modern-day synthetic fixation are used as fertilizer for agricultural production. However, much greater quantities of fertilizer are added to soil than is required by crops, and only ~50% of this N is incorporated into plant and microbial biomass or soil aggregates (Duan & Xiao 2000). The remaining fertilizer N is susceptible to ammonia volatilization, where large amounts of ammonia gas may be introduced to the atmosphere. Once in the atmosphere, this N can be transported long distances from where it originated (Liu et al. 2022). Reactive N in the atmosphere can be dissolved in precipitation and deposited across the landscape in rain and snow (wet deposition) or be deposited as aerosols or dust particles (dry deposition, which is mainly important in regions with low precipitation) (Dentener et al. 2006). Additionally, it has been shown that increasing rates of animal husbandry and livestock production are responsible for a substantial portion of the increase in atmospheric ammonia gas (Truong et al. 2018). The other major contributor to atmospheric N pollution is fossil fuel combustion (Wick et al. 2005). During the burning of fossil fuels,
nitrogen compounds may become oxidized, increasing nitrogen oxide emissions to the atmosphere (Fang et al. 2011). Overall, the deposition of biologically active forms of N onto land via the atmosphere has more than doubled since the pre-industrial period as a result of these anthropogenic sources (Tian et al. 2022). Not only may reactive forms of N be carried regionally via the atmosphere, but regions dominated by both industrial and agricultural activity tend to be hotspots for N pollution and subsequent deposition (Fang et al. 2011; Galloway et al. 2008).

*Spatial and temporal variation in nitrogen deposition effects*

Early successional habitats are often characterised by harsh conditions and low nutrient availability; N fixing species tend to be favoured in these environments and can play an important role in the progression to later successional stages (Sparrius et al. 2012). Nitrogen deposition can accelerate these early successional stages, increasing the abundance of later successional species such as graminoids and non-leguminous forbs (Huang et al. 2013). These species interactions can cause early successional habitats such as grasslands, young forests, and old fields to be impacted to a greater severity than later successional habitats such as old growth forests. Furthermore, the encroachment of woody species into early successional habitats can increase under elevated levels of N deposition (Archer et al. 2017). Northern temperate and arctic ecosystems are limited primarily by N and are anticipated to experience the greatest impacts of N deposition, whereas the ecosystems of tropical regions, located close to the equator, tend to be limited more by phosphorus, due to their ancient weathered soils and longer history of N fixation, and are more resilient to the effects of N deposition (Ellsworth et al. 2022).
Short- vs. long-term effects of nitrogen deposition

Because most terrestrial ecosystems are N limited (LeBauer & Treseder 2008; Vitousek & Howarth 1991), the availability of reactive N in these ecosystems is the governing factor controlling species composition (Craig & Henry 2022; Frey et al. 2023), along with ecosystem productivity and functioning (Keller et al. 2022; Vitousek et al. 1997). Nitrogen deposition often increases ecosystem primary productivity in the short term (Compton et al. 2013, Kondo et al. 2022, Ma et al. 2011), which can lead to increased investments of plants into rhizosphere compounds that can further increase microbial activity (Chen et al. 2015) and increase immobilization of N in plant and soil microbial biomass (Berntson & Aber 2000; Shindo & Nishio 2005). However, with chronic N deposition, N inputs can exceed plant and microbial N demand, shifting the ecosystem from N limited to N saturated (Brumme & Khanna 2008, Liu et al. 2017, Sheng et al. 2019) and diminishing its capacity to retain additional reactive N inputs (Hu et al. 2022; Liu et al. 2021). When ecosystems can no longer efficiently recycle N, nitrogen retention decreases and losses tend to increase through the stimulation of nitrification, and the loss of dissolved inorganic and organic N through hydrological processes (Oertel et al. 2016; Krol et al. 2016). The threshold of N inputs that exceed an ecosystem’s capabilities of recycling N and shifting to N saturation was recently estimated for global terrestrial ecosystems at 5 – 6 g N m⁻² y⁻¹ (Gurmesa et al. 2022; Tian et al. 2016), although this threshold can be influenced by additional factors such as ecosystem type and climatic conditions (Contosta et al. 2011, Marty et al. 2020, Zhang et al. 2020). Chronic N deposition also can contribute to soil acidification, eutrophication, and loss of biodiversity within a terrestrial ecosystem or to neighbouring ecosystems (Bai
et al. 2010, Silvertown et al. 2006, Stevens et al. 2004). Studies recording the effects of N deposition on plant and soil communities for over a decade have reported increased rates of N mineralization (Nave et al. 2009; Verma & Sagar 2020), paired with suppression of N immobilization; increased reactive N inputs into soil rapidly becomes susceptible to losses, and these increased N losses have been attributed to newly added sources of N, not from older reservoirs (Leimer et al. 2016, Song et al. 2019, Zhang et al. 2012).

Nevertheless, relatively few researchers have explored ecosystem responses to N deposition over long time scales (>10 years) (Komatsu et al. 2019). Not only can the effects of chronic N deposition on plants and soils be cumulative, but lags in changes in plant species composition can delay treatment effects in N addition experiments (Parmesan 2006).
Figure 1.1 Inputs, outputs, and internal cycling of nitrogen in an ecosystem.
Climate warming

Due to substantial increases in greenhouse gas emissions over the past century, global climate change has accelerated substantially (He & Li 2019; Heimann & Reichstein 2008), with elevated global air and ocean temperatures, melting of snow and ice, especially in polar regions, and rising sea levels (IPCC 2023). Global mean air temperature is projected to increase by 1.8 – 4°C relative to the pre-industrial period by the end of the 21st century, with warming intensifying with increasing latitude (IPCC 2023). Climate warming is anticipated to have significant impacts on terrestrial ecosystem functioning, because temperature impacts many biogeochemical processes, such as litter decomposition (Aerts 2006; Petraglia et al. 2019), N mineralization and nitrification (Sierra 1997; Zak et al. 1999), denitrification (Malhi et al. 1990; Zhang et al. 2022), plant nutrient uptake (BassiriRad et al. 2000; Gill & Jackson 2000), soil respiration (Raich & Schlesinger 1992), fine root dynamics (Pregitzer et al. 2000, Gill & Jackson 2000; Fitzhugh et al. 2001), and plant productivity (Grace 1988; Rustad et al. 2001). Additionally, climate warming can reduce the time that soils remain frozen, accelerate plant green-up, and extend the growing season (Linderholm 2006; Vega et al. 2020), which can increase net primary productivity, resulting in increased N storage in plant tissue (Turner & Henry 2010). The immobilization of N in plant tissues decreases bioavailable forms of N in the soil (Turner & Henry 2009); high rates of plant productivity also increase the flow of carbon from plant biomass to soil (Kroeger et al. 2021; Zhang et al. 2021), increasing the microbial demand for N (Finzi et al. 2006). However, warming also can increase soil microbial activity, increasing the rate of soil N mineralization; thus, increases in soil mineral N pool with warming, coupled with longer
growing seasons, can increase plant N availability (Bai et al. 2013; Cowles et al. 2016). Furthermore, warming can impact soil N retention by reducing snow cover and increasing the frequency of soil freeze-thaw cycles, resulting in altered belowground plant and soil microbial activities (Kim et al. 2017).

**Snow season length and snow cover**

Warming in temperate regions over the next century is expected to decrease snow season length and reduce the area of snow cover (IPCC 2023). Moreover, as a consequence of reduced snow cover, which decreases the insulation of the soil from cold air, winter warming can increase the frequency and intensity of soil freeze-thaw cycles (Mellander et al. 2007; Henry 2008), which in turn can increase N leaching losses due to microbial lysis (Yanai et al. 2004; Larsen et al. 2002), the disruption of soil aggregates (Fitzhugh et al. 2001; Six et al. 2004a), or damage to fine roots (Schimel & Clein 1996; Tierny et al. 2001). Increased soluble N accumulation in soil due to winter warming may also increase N trace gas emissions (Christensen & Tiedje 1990; Chang & Hao 2001). Temperate systems may be particularly sensitive to increased freeze-thaw cycles because their soils often remain close to the freezing point over much of the winter (Henry 2008).

**Short- vs. long-term effects of warming on community dynamics and succession**

Multiple studies have documented that significant changes in the responses of grassland communities to warming may take at least a decade to emerge (Hu et al. 2021; Komatsu et al. 2019). Warming may rapidly increase plant community productivity in the
short term, however longer-term warming treatments can influence community successional patterns in terrestrial ecosystems. Guo et al. (2018) observed an increasingly divergent succession of microbial communities based on long term climate warming projections, with potential higher impacts on fungi compared to bacteria, while Shi et al. (2018) observed a transition from an annual C-3 dominated plant community to a perennial C-4 dominated plant community in 8 years. Old fields in particular can reach later successional stages much faster by favouring more competitive generalist species that can quickly colonise a field (George et al. 2009), i.e. old field ecosystems follow a fairly predictable pattern from weedy annual herbaceous species being replaced by perennials (Avolio et al. 2019; Huang et al. 2021), then woody encroachment and the addition of later successional trees and shrubs (Criado et al. 2020). It has therefore been suggested that, in the short-term, warming may mostly affect N retention through changes in N mineralization, whereas plant composition effects on N storage may be a bigger factor over the longer-term, such that warming may not decrease soil N availability over time (Gao & Yan 2019). It also has been suggested that microbial communities can develop more efficient nitrogen and carbon recycling mechanisms over time (Bradford & Fierer 2012; Hirel et al. 2011). Overall, many ecosystem responses to global change may be realized only over longer time-scales (Nottingham et al. 2019; Six et al. 2004b).

**Interannual variation in weather**

Despite the potential emergence of cumulative, long-term effects in global change field experiments, the comparison of the early years of a field experiment with later years nevertheless can be confounded by interannual variation in weather or other factors. Such
variation could affect both the strength and direction of plant responses to experimental warming and N deposition treatments (Henry et al. 2015). For example, in temperate grasslands, annual variation in precipitation and temperature have large effects on net ecosystem carbon storage (Novick 2004). Likewise, across a precipitation gradient, plant inputs of carbon and nitrogen into soils increased with increasing precipitation (Wang et al. 2015), which influenced both labile and recalcitrant pools of N; microbial net N mineralization and soil nitrification rates also increased with increasing precipitation (Feyissa et al. 2021).

**Old fields as a study system for global change**

Old fields are ecosystems that were previously human-managed, usually as agricultural fields used for growing crops or animal husbandry but have since been abandoned. Old field plant communities usually follow predictable patterns of succession to return to a state resembling their historical species composition; colonized first by annual grasses and forbs in the short term before shifting into a state with longer-lived perennial grasses, forbs, and leguminous-forb species over the longer term (Avolio et al. 2019; Huang et al. 2021). Finally, in temperate regions, these ecosystems usually undergo woody encroachment and eventually enter a steady state dominated by trees and shrubs (Criado et al. 2020). However, these ecosystems may have altered functioning and species composition due to years of nutrient inputs, biomass removal, and alterations to the soil profile and local hydrological cycles (Cramer et al. 2008, Dambrine et al. 2007, Foster et al. 2003, McLauchlan 2006). In addition, newly abandoned old fields can be colonized by
invasive species (Standish et al. 2008) that can persist for decades (Cramer & Hobbs 2007).

The early stages of old field succession can provide a useful system for studying the responses of grass-dominated ecosystems to global change (Prommer et al. 2020). Grass-dominated systems are highly tractable for administering treatments at the plot level in field experiments because of the size of the plants. Moreover, grass-dominated systems are globally relevant, being the most extensive terrestrial biome on Earth (Gibson & Newman 2019), covering 50 million square kilometers and making up 37% of the planet’s terrestrial area (O’Mara 2012). Furthermore, N cycling responses to global change have been studied extensively in grass-dominated plant communities.

In situ measurements of nitrogen mineralization and leaching

The estimation of soil net N mineralization provides useful insight into the availability of N for use by plants. Several techniques have been developed for in situ measurements of soil N availability. The field technique commonly known as the “buried bag” method involves burying polyethylene bags of soil, and comparing the amount of inorganic N at the beginning of the incubation to the amount present at the end (e.g., an increase would indicate net mineralization). These bags allow for the diffusion of gases through the bag but do not allow the transfer of liquids (Gordon et al. 1987). However, this method introduces an experimental artefact where soil water dynamics at the start of the incubation must reflect conditions throughout the entirety of the incubation (Isaac & Timmer 2007). Otherwise, key interactions between nutrient cycling and hydrological processes will not be accounted for. The “covered core” method, a modification of the
buried bag method, allows for increased aeration of the soil, but still hinders vertical movement of water through the core (Adams et al. 1989). This has led to the development of the ion exchange resin core method (Isaac & Timmer 2007), which allows the vertical movement of both gases and liquids. With the placement of ion exchange resin at the bottom of the core, dissolved mineral N leached out of the core can be quantified. Similarly, ion exchange resin placed at the top of the core, and a second bottom layer located below the leachate-ccatching resin, prevent unwanted N contamination from entering into the soil core.

*The use of stable isotopes to assess ecosystem nitrogen retention*

The use of stable isotopes of nitrogen is used extensively for tracing the movement and retention of N in biological systems (Hou et al. 2015). The stable nitrogen isotopes of masses 14 and 15 (\(^{14}\text{N}\) and \(^{15}\text{N}\)) exist naturally in an almost constant ratio of \(~272:1\) (\(^{14}\text{N} : ^{15}\text{N}\)), otherwise described as a natural occurrence of \(^{15}\text{N}\) of 0.363%. Stable isotope tracer techniques artificially enrich a system with the rarer heavy isotope, by adding a known amount of \(~100\%\) \(^{15}\text{N}\) enriched substrate (Hauck 1976). The amount of \(^{15}\text{N}\) collected in any subsequent samples can be compared to the initial amounts to determine the relative retention of N in various pools (plant and soil), with total N and \(^{15}\text{N}\) determined in samples through mass spectroscopy analysis. Percent \(^{15}\text{N}\) by mass in the soil and plant samples can be determined, with the tracer \(^{15}\text{N}\) being determined by subtracting the natural occurring amount from the total \(^{15}\text{N}\) by mass.
1.2 Objectives and Hypotheses

The goal of my research was to compare the short-term (<3 years) versus long-term effects (>16 years) of N addition and warming on the N dynamics of a temperate grass-dominated old field. By comparing the responses of a set of treatment plots established 16 years ago to those of newer plots established at the same location, I was able to compare the short- versus long-term effects independent of interannual variation in background environmental conditions. My specific objectives were as follows.

Objective 1) To compare the short- vs. long-term effects of N addition and warming on soil N mineralization and leaching.

I used *in situ* soil cores to examine net N mineralization responses to N addition and warming over one year (3 incubations: spring to peak growing season, peak growing season to first snowfall, over winter). I also quantified N leaching losses from the soil cores using ion exchange resin placed at the bottom of each soil core. I predicted that both N addition and warming would increase N mineralization, and that these effects would be additive. However, I predicted the treatments would have contrasting effects on N leaching, with increased N leaching in the N addition plots and decreased N leaching in the warmed plots. I predicted the negative effects of N addition on N leaching would outweigh the positive effects of warming, and treatment effects on both N mineralization and N leaching would be greater for the long-term plots than in the short-term plots.
Objective 2) To compare the short- versus long-term effects of N addition and warming on plant and soil N retention.

I added $^{15}$N-labeled ammonium nitrate tracer to the soil surface prior to plant green-up, then determining the quantity of $^{15}$N in excess of natural abundance in both the aboveground grass biomass and soil at the peak of the growing season. I predicted N addition would decrease $^{15}$N tracer retention, whereas warming would increase $^{15}$N tracer retention. I predicted the negative effects of N addition would outweigh the positive effects of warming, and there would be less $^{15}$N tracer retention in the long-term plots compared to in the short-term plots.

1.3 Thesis Organization

My thesis is organized in the integrated article format and contains two data chapters. Chapter 1 provides a general introduction with relevant background information and an overview of the experimental approaches used to address my research questions. The first data chapter (Chapter 2) addresses my first research objective by presenting the results from my $in situ$ soil core net N mineralization and leaching analyses. The second manuscript (Chapter 3) addresses my second research objective by presenting the results from my $^{15}$N tracer analyses. The final chapter (Chapter 4) discusses and connects the results of the two data chapters and provides insight for potential future research directions.
1.4 References


terrestrial biosphere: a 5 arcmin resolution annual dataset from 1860 to 2019.

*Earth System Science Data*, 14, 4551 – 4568.


Chapter 2

Short- versus long-term effects of nitrogen addition and warming on soil nitrogen mineralization and leaching

2.1 Introduction

Temporal scale can be a key aspect of interpreting the responses of soil nitrogen dynamics to global change. For many terrestrial ecosystems, the deposition of reactive nitrogen (N) from the atmosphere can have immediate effects in the short-term by increasing plant productivity (Vitousek & Howarth 1991) and net N mineralization rates (Hu et al. 2021b). However, chronic atmospheric N deposition can exceed plant and microbial demand for N over the longer term and shift ecosystems from being N limited to N saturated (Tian et al. 2016). Soil N not incorporated into plants, microbes or soil aggregates can accumulate in the soluble N pool, and is susceptible to increased hydrological losses and trace gas emissions (Verma & Sagar 2020). Climate warming is another global change factor that can stimulate plant growth and N incorporation by reducing the time that soils remain frozen, accelerating plant green-up and extending the growing season (Vega et al. 2020). Moreover, by stimulating soil microbial activity, warming can increase soil N availability in the short term (Sun et al. 2022). Nevertheless, longer-term shifts in plant community composition in response to warming can have additional effects on soil N availability (Hu et al. 2021a).

The short term (i.e. 1-3 year) responses of soil N mineralization to N addition and warming in field experiments have been reported extensively. For example, warming and N addition can alter many aboveground processes through changes to plant productivity...
(Yue et al. 2020), influences on plant N concentrations (Hu et al. 2022), and the quality of litter shed from senescing plant tissues (Henry et al. 2015). Moreover, N additions and warming can alter plant community composition (Zhang et al. 2015), and influence structure and functioning by favouring certain functional types over others (Zhao et al. 2019). Furthermore, N addition and warming can have substantial impacts on belowground microbial communities by increasing mineralization rates (Marty et al. 2020), altering microbial biomass stoichiometry (Gong et al. 2019), accelerating decomposition rates (Gong et al. 2015), and may affect soil N losses by increasing N leaching and nitrous oxide (N\textsubscript{2}O) emissions (Zhang et al. 2020; Zhang et al. 2022).

Regarding longer term responses, N addition treatments can steadily increase plant productivity for up to several decades (Liang et al. 2021). Similarly, there is evidence from field experiments that warming for over a decade can lower soil N stocks (Phillips et al. 2019) and accelerate N mineralization rates (Zhang et al. 2021), and that both long-term warming and N addition can increase soil N losses (Frey et al. 2023). Accumulation of plant derived N in soils that are not becoming incorporated in microbial biomass due to decreased litter decomposition, microbial N demand, and N cycling (Biudes et al. 2013) can increase soil N leaching losses (Fang et al. 2009), especially during snow melt and at times with large fluxes of water moving through a system (Yano et al. 2015). However, relatively few experiments have examined the effects of N addition and warming on soil N dynamics over the time scale of decades.

Although long-term responses of soil N to global change can be driven by cumulative changes in soil N (Six et al. 2004) or by changes in plant relative abundances or community composition (Komatsu et al. 2019), in field experiments, these responses also can be influenced by background shifts in environmental conditions that do no
remain consistent over the course of the experiment (He & Li 2019), which could interact with the global change treatments. For example, in an N addition field experiment conducted by Hutchison & Henry (2010), the suppression of plant biomass responses to N addition during the initial years of the study were attributed to drought. It follows that it cannot be concluded with certainty that the comparison of soil N mineralization measured during the later years of a field experiment with measurements obtained in the earlier years represents a long-term effect.

I compared the short-term versus long-term effects of N addition and warming on soil N mineralization and leaching in a grass-dominated old field. In addition to the original N addition and warming experiment that had been established for 16 years, a newer set of N addition, warming and control plots, interspersed among the original plots, was established for the last three years of the experiment. The advantage of this design was that the short- and long-term effects could be compared while controlling for possible variation in background environmental conditions. I predicted that both the N addition and the warming treatments would increase N mineralization and that these effects would be additive. However, I also predicted the treatments would have contrasting effects on N leaching, with increased leaching in the N addition plots and decreased leaching in the warmed plots. Regarding the short- versus long-term effects, I predicted the N mineralization and leaching effects would be intensified in the long-term plots.
2.2 Methods

2.2.1 Site description

The field experiment was established in a grass-dominated temperate old field, located at the Agriculture Canada Southern Crop Protection and Food Research Centre in London, Ontario, Canada (43°01´46˝N, 81°12´52˝W). This former agricultural field was cropped previously with rotations of wheat, corn, and soybean, then taken out of production and seeded with grasses over 35 years ago. The field was dominated by the perennial cool season grasses Poa pratensis L. and Bromus inermis Leyss. Also present at the field site were the clonal perennial forbs species Cirsium arvense L., Asclepias syriaca L., Aster ericoides L., and Solidago altissima L., and the legume Lotus corniculatus L. (Hutchison & Henry 2010). The soil at the site was classified as well to imperfectly drained silt loam glacial till (Hagerty & Kingston 1992) with a composition of 9% clay, 41% silt, and 50% sand, and it had an average pH of 7.6 (Bell et al. 2010). Mean annual air temperature at the field site over the study period (from April 2022 - April 2023) was 9.3 °C, which was warmer than the average mean air temperature of 8.6°C, and annual precipitation was 746 mm over the study period, which was drier than the average annual precipitation of 1055 mm for the region (1981-2010 Climate Normal Data, Environment Canada, National Climate Data and Information Archive).

2.2.2 Warming and N addition treatments

The plots of the experiment were organized in a randomized block design (n = 10) with two warming treatments (warming year-round and no warming) and two N addition treatments (nitrogen fertilization and ambient nitrogen). While the long-term treatment
plots, established in late 2006, included control, N-only, warming only, and combined N and warming plots (40 plots total), the short-term treatment plots, established in late 2019, included only control, N-only, and warming plots (30 plots total), and did not include combined N and warming plots (Figure 2.1). Each plot was circular (1.13 m in diameter) and the treatments extended 10 cm further outwards into a treated buffer zone. Warming was administered at the plot-level with 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) suspended 50 cm above the soil. Ceramic heaters warm the plants and soil without producing photosynthetically active radiation (Harte et al. 1995). Nitrogen addition was administered as a pulse of aqueous NH₄NO₃ added in late March each year at a rate of 2 g m⁻² y⁻¹, and slow release NH₄NO₃ pellets (Florikan ESA, Sarasota, Fla.) were added in late May at a rate of 4 g m⁻² y⁻¹. The total rate of 6 g m⁻² y⁻¹ was consistent with the high-end estimates for N deposition rates in 2050, as described by Galloway et al. (2004). Soil temperature was recorded hourly by underground temperature probes (107-BAM, Campbell Scientific Canada Corp., Edmonton, AB, Canada) located at 2 and 5 cm depth, and soil moisture was also recorded hourly with soil moisture probes (CS616-L TDR probes, Campbell Scientific Canada Corp., Edmonton, AB, Canada) located at 0-15 cm and 0-30 cm depths. However, the corruption of datalogger files precluded the recovery of soil temperature and moisture data over much of the 2022-2023 study period when the soil N analyses were conducted.
\[ \text{Plot} \]

- \(\bullet\) = IR heater
- \(\circ\) = dummy heater
- 16 = old plot (16 years)
- 3 = new plot (3 years)

1 m
Figure 2.1 Plot design, showing a representative block (1 of 10). Plots consisted of short-term (<3 years) treatments, including control plots (C) and those receiving either N addition (N) or warming (W), and long-term (>16 years) treatments, including control plots (C) and those receiving either N addition (N), warming (W) or both N addition and warming combined (NW). Other plots, that were not used in this study, are denoted by an X.
2.2.3 Net N mineralization and leaching

I estimated net N mineralization using the ion exchange resin core method (Isaac & Timmer 2007). I collected soil cores inside 4.3 cm × 15 cm PVC tubes and incubated a series of these cores in situ over one year. Ion exchange resin bags, containing mixed cation/anion exchange resin beads (Dowex Marathon MR-3 mixed bed resins, hydrogen and hydroxide form), were activated by soaking the resins in 5 M NaCl, with a small amount of NaOH added, overnight. I placed activated resin bags at both the top and bottom of the soil core to intercept and deionize water entering into the core (Figure 2.2). I also placed a second inner resin bag at the bottom of each core to capture drainage and dissolved solutes leaching out of the soil core (Meißner et al. 2010). A subsample of soil was collected adjacent to each core at the start of the incubation to measure the initial inorganic soil N.

The effects of N addition and warming in field experiments can vary substantially by season (Contosta et al. 2011). Therefore, I ran three soil core incubations in series over the course of a year (Figure 2.3). During the first incubation, I incubated the soil core over the growing season (April 2022 - July 2022), then removed the soil core, homogenized it, and collected a 15 g subsample. I extracted each soil subsample in 2 M KCl for 1 h, with the extract filtered through pre-leached Whatman No. 1 filter paper and frozen within 24 h for future colorimetric analysis of nitrate and ammonium concentrations (Klute 1986). I conducted the latter analyses using a discrete analyser (SmartChem 140, Unity Scientific). The contents of the inner resin bag were also extracted in 2 M KCl for 30 minutes and analysed using the discrete analyser to measure ammonium and nitrate leached out of the column. Net N mineralization was estimated by
**Figure 2.2.** Design of the *in situ* soil cores for quantifying N mineralization and leaching. Resin bags placed at the top and bottom of the core intercepted contamination from entering the core, while the inner bottom resin bag captured dissolved NH$_4^+$ and NO$_3^-$ leaching through the core. Soil and resin extractable N were compared to initial N quantities, adjusting for the leaching losses, to estimate net N mineralization over the incubation period.
comparing the ammonium and nitrate content of the soil samples after incubation plus the leached N to the ammonium and nitrate content measured prior to incubation. I also incubated soil cores from July 2022 to November 2022, and from November 2022 to April 2023, following the same procedure as the growing season incubation. Each time, the resin bags were re-soaked in the activation solution to recharge their ion exchange capacity for subsequent use.
Figure 2.3 The start (down arrows) and end (up arrows) of incubation periods for the mineralization cores, plotted against A) daily mean ambient air temperature, B) daily precipitation, and C) snow cover present at the field site from 1 October 2021 through 31 May 2023. In C), the black vertical lines indicate the presence of snow cover on the plots (C - ambient temperature plots; W – warmed plots).
2.2.4 Statistical analyses

Given that the experimental design was not completely factorial (i.e. there were no short-term plots with the combination of N addition and warming), I used three separate two-way ANOVAs to examine the following: 1) the effects of N addition, plot age, and their interaction, 2) the effects of warming, plot age, and their interaction, 3) the effects of N addition, warming and their interaction in the old plots only. The response variables I examined for each incubation period (1 - from spring to peak growing season, 2 - from peak growing season to first snowfall and 3 - over winter) were changes in soil extractable inorganic N, resin extractable inorganic N, and total N mineralization (i.e. the sum of changes in soil extractable N and resin extractable N). For each analysis, block was included in the ANOVA model as a random factor to take into account possible variation among blocks. Resin extractable inorganic N values were log-transformed to achieve normality and homogeneity of variances.

2.3 Results

2.3.1 Net N mineralization and leaching from spring to peak growing season

For total N mineralization from spring to mid-summer, there was an interaction between N addition and age (P_{N*Age}=0.044, Table 2.1, Fig. 2.4A), explained by there being decreased N mineralization with N addition, but only in the new plots (Fig. 2.4A). This effect was driven by changes in extractable mineral N in the soil cores, which were much higher than leaching losses from the cores at this time (Fig. 2.4B, C). Net immobilization of NO_3^- was observed in the old control plots combined with ~11 times less resin extractable NO_3^- when compared to the old N addition plots (Table 2.2).
**Figure 2.4 A)** Total N mineralization/immobilization (B + C), **B)** resin extractable N, and **C)** changes in soil extractable N (soil extractable after incubation – soil extractable prior to incubation) from spring to peak growing season (13 April 2022 to 11 July 2022). Resin extractable N data was log$_{10}$ transformed for statistical analyses then back transformed for display in the figures. Means and standard errors (n=10) are presented with i) comparing the N addition treatment with the control in both the short- and long-term plots, ii) comparing the warming treatment with the control in both the short- and long-term plots, and iii) comparing the N addition, warming, and combined treatments with the control in the long-term plots only. Positive values for changes in soil extractable N and total N mineralization show net mineralization whereas negative values show net immobilization of N. Significant values (P<0.05) are displayed on the panels.
Table 2.1 P values determined by three two-way ANOVAs testing for significant effects of 1) plot age, N addition, and their interaction, 2) plot age, warming, and their interaction, and 3) N addition, warming, and their interaction on change in soil extractable inorganic N, resin extractable N, and total mineralization of N (the combination of soil and resin extractable inorganic N) over all three incubation periods. Values in bold indicate a significant treatment effect.

<table>
<thead>
<tr>
<th></th>
<th>Growing season</th>
<th>Peak growing season - first snowfall</th>
<th>Over winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total N</td>
<td>Resin N</td>
<td>Change N</td>
</tr>
<tr>
<td><strong>Old + New plots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.776</td>
<td>0.262</td>
<td>0.999</td>
</tr>
<tr>
<td>N</td>
<td>0.539</td>
<td>0.084</td>
<td>0.330</td>
</tr>
<tr>
<td>N * Age</td>
<td><strong>0.044</strong></td>
<td>0.228</td>
<td>0.062</td>
</tr>
<tr>
<td><strong>Old + New plots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.124</td>
<td>0.187</td>
<td>0.141</td>
</tr>
<tr>
<td>W</td>
<td>0.771</td>
<td>0.157</td>
<td>0.896</td>
</tr>
<tr>
<td>W * Age</td>
<td>0.888</td>
<td>0.220</td>
<td>0.765</td>
</tr>
<tr>
<td><strong>Old plots only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.262</td>
<td>0.114</td>
<td>0.478</td>
</tr>
<tr>
<td>W</td>
<td>0.967</td>
<td>0.605</td>
<td>0.877</td>
</tr>
<tr>
<td>N * W</td>
<td>0.656</td>
<td>0.519</td>
<td>0.708</td>
</tr>
</tbody>
</table>
2.3.2 Net N mineralization and leaching from peak growing season to first snowfall

Over the late summer and fall, there was an increase in total N mineralization in the new warmed plots, but not in the old warmed plots (Fig. 2.5A), which was driven by changes in extractable N (Fig. 2.5A), but this interaction between warming and plot age was only marginal ($P_{W \times Age} = 0.063$, Table 2.1). In response to N addition, there was a decrease in extractable N over this period ($P_N=0.013$, Table 2.1, Fig. 2.5C), but in the old plots, this decrease did not occur in combination with warming ($P_{N \times W}=0.049$, Table 2.1, Fig. 2.5C). N leaching also increased in response to N addition ($P_N=0.031$, Table 2.1, Fig. 2.5B), but in the old plots alone, this N addition effect ($P_N=0.016$) did not interact with warming ($P_W=0.83$, Table 2.1, Fig. 2.5B). N leaching was predominately in the form of nitrate (Table 2.2).
Table 2.2 The change in soil extractable inorganic nitrate and ammonium, resin extractable inorganic nitrate and ammonium, and total mineralization of nitrate and ammonium (soil and resin extractable inorganic nitrate and ammonium) for each incubation period expressed per unit of soil dry weight (μg g⁻¹ dw). Incubation periods were over the growing season (April 2022 – July 2022), from the time of peak biomass until first snowfall (July 202 – November 2022), and over winter (November 2022 – April 2023). Standard errors for each mean are provided in parentheses.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial NH₄⁺</th>
<th>Final NH₄⁺</th>
<th>Resin NH₄⁺</th>
<th>Initial NO₃⁻</th>
<th>Final NO₃⁻</th>
<th>Resin NO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growing season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old control</td>
<td>2.26 (0.23)</td>
<td>8.76 (0.68)</td>
<td>0.19 (0.03)</td>
<td>1.08 (0.13)</td>
<td>0.82 (0.18)</td>
<td>0.06 (0.03)</td>
</tr>
<tr>
<td>Old N</td>
<td>2.80 (0.27)</td>
<td>9.60 (0.92)</td>
<td>0.22 (0.03)</td>
<td>1.13 (0.09)</td>
<td>1.58 (0.45)</td>
<td>0.68 (0.48)</td>
</tr>
<tr>
<td>Old warming</td>
<td>2.12 (0.31)</td>
<td>8.78 (1.13)</td>
<td>0.19 (0.03)</td>
<td>0.92 (0.20)</td>
<td>1.03 (0.23)</td>
<td>0.08 (0.04)</td>
</tr>
<tr>
<td>Old combined</td>
<td>2.95 (0.43)</td>
<td>9.94 (0.84)</td>
<td>0.22 (0.04)</td>
<td>1.44 (0.32)</td>
<td>1.66 (0.51)</td>
<td>0.44 (0.36)</td>
</tr>
<tr>
<td>New control</td>
<td>2.66 (0.32)</td>
<td>9.41 (0.91)</td>
<td>0.20 (0.02)</td>
<td>0.88 (0.13)</td>
<td>1.09 (0.25)</td>
<td>0.05 (0.02)</td>
</tr>
<tr>
<td>New N</td>
<td>3.67 (0.43)</td>
<td>8.89 (0.98)</td>
<td>0.21 (0.02)</td>
<td>1.34 (0.22)</td>
<td>1.37 (0.47)</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>New warming</td>
<td>2.07 (0.22)</td>
<td>10.71 (1.42)</td>
<td>0.23 (0.02)</td>
<td>0.87 (0.11)</td>
<td>1.61 (0.71)</td>
<td>0.38 (0.30)</td>
</tr>
<tr>
<td><strong>Peak growing season - first snowfall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old control</td>
<td>6.42 (0.25)</td>
<td>7.14 (0.16)</td>
<td>0.20 (0.02)</td>
<td>0.67 (0.09)</td>
<td>0.63 (0.19)</td>
<td>0.03 (0.02)</td>
</tr>
<tr>
<td>Old N</td>
<td>8.14 (0.32)</td>
<td>7.08 (0.19)</td>
<td>0.24 (0.02)</td>
<td>1.32 (0.23)</td>
<td>0.58 (0.10)</td>
<td>1.14 (0.81)</td>
</tr>
<tr>
<td>Old warming</td>
<td>6.93 (0.43)</td>
<td>6.88 (0.13)</td>
<td>0.22 (0.01)</td>
<td>0.69 (0.09)</td>
<td>0.70 (0.27)</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td>Old combined</td>
<td>7.40 (0.27)</td>
<td>7.28 (0.21)</td>
<td>0.22 (0.01)</td>
<td>1.37 (0.30)</td>
<td>1.21 (0.87)</td>
<td>0.09 (0.04)</td>
</tr>
<tr>
<td>New control</td>
<td>6.91 (0.55)</td>
<td>7.47 (0.73)</td>
<td>0.25 (0.01)</td>
<td>0.64 (0.07)</td>
<td>0.38 (0.13)</td>
<td>0.17 (0.16)</td>
</tr>
<tr>
<td>New N</td>
<td>7.51 (0.36)</td>
<td>7.16 (0.19)</td>
<td>0.21 (0.02)</td>
<td>1.12 (0.30)</td>
<td>0.45 (0.13)</td>
<td>1.09 (0.71)</td>
</tr>
<tr>
<td>New warming</td>
<td>6.37 (0.13)</td>
<td>6.92 (0.13)</td>
<td>0.21 (0.01)</td>
<td>0.61 (0.08)</td>
<td>2.66 (1.57)</td>
<td>0.35 (0.31)</td>
</tr>
<tr>
<td><strong>Over winter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old control</td>
<td>7.58 (0.35)</td>
<td>2.73 (0.64)</td>
<td>0.19 (0.08)</td>
<td>0.43 (0.05)</td>
<td>0.57 (0.09)</td>
<td>0.50 (0.21)</td>
</tr>
<tr>
<td>Old N</td>
<td>8.65 (0.66)</td>
<td>3.93 (1.17)</td>
<td>0.13 (0.02)</td>
<td>0.74 (0.19)</td>
<td>0.77 (0.09)</td>
<td>2.72 (0.94)</td>
</tr>
<tr>
<td>Old warming</td>
<td>7.77 (0.13)</td>
<td>3.55 (0.91)</td>
<td>0.11 (0.03)</td>
<td>0.35 (0.05)</td>
<td>0.50 (0.07)</td>
<td>0.52 (0.24)</td>
</tr>
<tr>
<td>Old combined</td>
<td>7.95 (0.29)</td>
<td>3.46 (0.95)</td>
<td>0.26 (0.07)</td>
<td>0.59 (0.12)</td>
<td>0.73 (0.13)</td>
<td>3.18 (1.09)</td>
</tr>
<tr>
<td>New control</td>
<td>7.38 (0.34)</td>
<td>2.47 (0.64)</td>
<td>0.12 (0.04)</td>
<td>0.33 (0.03)</td>
<td>0.54 (0.10)</td>
<td>1.09 (0.55)</td>
</tr>
<tr>
<td>New N</td>
<td>8.83 (0.96)</td>
<td>2.21 (0.71)</td>
<td>0.25 (0.08)</td>
<td>0.95 (0.48)</td>
<td>0.58 (0.08)</td>
<td>1.89 (0.95)</td>
</tr>
<tr>
<td>New warming</td>
<td>7.64 (0.30)</td>
<td>1.75 (0.58)</td>
<td>0.18 (0.08)</td>
<td>0.35 (0.01)</td>
<td>0.49 (0.08)</td>
<td>0.43 (0.17)</td>
</tr>
</tbody>
</table>
Figure 2.5 A) Total N mineralization/immobilization (B + C) B) resin extractable N, and C) changes in soil extractable N (soil extractable after incubation – soil extractable prior to incubation) from peak growing season to first snowfall (12 July 2022 to 14 November 2022). Resin extractable N data was log_{10} transformed for statistical analyses then back transformed for display in the figures. Means and standard errors (n=10) are presented with i) comparing the N addition treatment with the control in both the short- and long-term plots, ii) comparing the warming treatment with the control in both the short- and long-term plots, and iii) comparing the N addition, warming, and combined treatments with the control in the long-term plots only. Positive values for changes in soil extractable N and total N mineralization show net mineralization whereas negative values show net immobilization of N. Significant values (P<0.05) are displayed on the panels.
2.3.3. *Net N mineralization and leaching over winter*

There were no significant interactions between either of the treatments and plot age over winter. However, in response to N addition there was decreased N immobilization; this effect was significant for the old plots analysed in isolation (P<sub>N</sub>=0.018, Table 2.1, Fig. 2.6A), and also marginally significant for the old and new plots combined (P<sub>N</sub>=0.087, Table 2.1, Fig. 2.6A). The decreased N immobilization in the N addition plots was accompanied by an increase in N leaching losses (P<sub>N</sub><0.001, Table 2.1, Fig. 2.6B), and the main form of N lost through leaching was nitrate (Table 2.2). On balance, with decreased N immobilization and increased N leaching in the N addition plots, there was no significant N addition effect on the change in extractable N (Fig. 2.6C). However, there was a significant interaction between plot age and warming for the change in extractable N (P<sub>W*Age</sub>=0.045, Table 2.1), caused by there being less of a reduction in extractable N in the new warmed plots compared to in the new control plots (Fig. 2.6C).
**Figure 2.6 A)** Total N mineralization/immobilization (B + C), **B)** resin extractable N, and **C)** changes in soil extractable N (soil extractable after incubation – soil extractable prior to incubation) over winter (15 November 2022 to 6 April 2023). Resin extractable N data was log$_{10}$ transformed for statistical analyses then back transformed for display in the figures. Means and standard errors (n=10) are presented with i) comparing the N addition treatment with the control in both the short- and long-term plots, ii) comparing the warming treatment with the control in both the short- and long-term plots, and iii) comparing the N addition, warming, and combined treatments with the control in the long-term plots only. Positive values for changes in soil extractable N and total N mineralization show net mineralization whereas negative values show net immobilization of N. Significant values (P<0.05) are displayed on the panels.
2.4 Discussion

I predicted that N addition would increase N mineralization, and that this effect would be greatest in the old plots. Over the spring to the peak of the plant growing season, both the interaction between plot age and N addition and the response of increased N mineralization with N addition in the old plots were indeed consistent with my prediction. However, the opposite effect of decreased N mineralization with N addition in the new plots was contrary to my prediction. The latter result suggests that N remained limiting in the new N addition plots in the short term, which was supported by the responses of primary productivity to N addition documented in a previous study at the site (Craig & Henry 2022). In contrast, the microbial demand for N following the breakdown of soil organic matter would be low in the old N addition plots, consistent with soil N saturation and elevated levels of extractable inorganic N (Gurmesa et al. 2022). Notably, the N mineralization response to N addition in the new plots from spring to mid summer was contrary to what was observed in the early years of the initial experiment, when the plots designated as old in the current experiment were only a couple of years old; at that time, there was no significant effect of N addition on total N mineralization (Turner & Henry 2010). This inconsistency highlights the value of my approach, which controlled for the effects of background variation in environmental conditions when comparing between the old and new plots. In particular, the N mineralization analyses conducted over a decade earlier on the old plots when they were the same age as the current new plots coincided with a summer drought, which may explain the lack of a significant effect of N addition on total N mineralization in that year. Regarding warming effects, although I predicted in the current study that warming would
increase N mineralization and there would be an interaction with plot age, there were no significant effects of warming on N mineralization over summer, which was consistent with the earlier years of the experiment (Turner & Henry 2010).

In the late summer and fall, there was an overall reduction in total N mineralization relative to earlier in the summer, but the continuation of relatively high N mineralization in the new, warmed plots matched my prediction of increased N mineralization with warming. However, the reduced warming effect in the old plots relative to in the new plots was contrary to my prediction. Nevertheless, reduced sensitivity of the soil microbial community to warming over time has been noted elsewhere for grassland systems in the context of soil respiration (Harte et al. 1995, Kroeger et al. 2021, Luo et al. 2001). Longer-term warming effects also could emerge in response to shifts in plant community composition, which often exhibit substantial time lags in grass-dominated systems and may not emerge until over a decade after treatments commence (Komatsu et al. 2019). However, in the current field experiment, continued dominance by the two most abundant grass species after over 16 years of warming and nitrogen treatments (Craig & Henry 2022) has diminished the potential role of a shift in plant species composition as a driver of soil responses. Although N addition led to increased grass biomass production in the experiment (Craig & Henry 2022; Gough 2011), large plants tend to incorporate disproportionately larger amounts of N in their tissues than smaller plants, this can be counterbalanced by increased root biomass turnover, which can increase soil available N in ecosystems that experience high N deposition (Keller et al. 2022).

My observation of increased N leaching losses over the late summer and fall (albeit relatively small in magnitude) was consistent with the frequent observation that N
addition promotes increased N losses (Lemke et al. 1998; Verma & Sagar 2020). However, contrary to my prediction, there was no difference in N addition effects on leaching at this time between the new and old plots. These increased N leaching losses with N addition coincided increased changes in soil extractable N at this time, which were also highest in the N addition plots, so on balance there was no significant effect on total N mineralization, but instead a difference in the fate of the mineralized N (i.e. lost vs. retained in the soil). Interestingly, the significant interaction between N addition and warming for the old plots revealed that the increased decrease in extractable N did not occur in the warmed plots, which would be consistent with increased microbial activity and N immobilization in response to warming (Nottingham et al. 2019).

Over winter, the large leaching losses in response to N addition were consistent with my prediction; nevertheless, while these losses were larger in the old plots than in the new plots, the interaction between plot age and N addition was not significant. Plant and soil microbial uptake of N slows substantially over winter, and soil freeze-thaw cycles can disrupt soil aggregates, lyse microbial cells, and damage fine roots, further increasing the size of the inorganic N pool (Juan et al. 2018, Sanders-DeMott et al. 2018, Xie et al. 2015). Thereafter, snow and ice melt can increase the flow of mineral N from soil to groundwater through leaching (Harms et al. 2019). Spring snow melt, which was included in the overwinter incubation period, coincided with particularly large fluxes of water and nutrients through the soil profile (Yoshitake et al. 2021). The observation of increased total N mineralization in response to N addition also was consistent with my prediction (albeit with the lack, again, of a significant N addition by plot age interaction). Unlike in the late summer and fall, the increased mineral N in the N addition plots was
matched by the increase in N leaching losses, such that on balance there was no significant effect of N addition of the change in extractable mineral N over winter.

Although the increased change in extractable N with warming observed over winter was consistent with my prediction, the fact that this only occurred in the new plots was not; as with the similar trend observed for the late summer and fall, this result indicates reduced sensitivity of the soil microbial community to warming over time (Gong et al. 2019; Tikariha et al. 2019). Warming can increase inorganic N by reducing snow cover and consequently increasing the frequency and intensity of soil freeze-thaw cycles (Juan et al. 2020). However, previous soil freezing experiments at this site indicate that only very extreme soil freeze-thaw cycles, larger than what is caused by the overhead heaters (Jospeh & Henry 2008), result in measurable changes to the soil extractable N. Instead, increased microbial activity and N mineralization caused by warming were likely responsible for the increases observed in the new plots.

2.5 Conclusions

Overall, my results demonstrate that comparing results from the early years of a global change experiment to those of later years does not necessarily provide an accurate measure of short-term versus longer-term effects on soil N processes. Moreover, contrary to my prediction, the difference between short- versus long-term effects is not simply one of treatment effects on soil N processes intensifying over the longer term. A key aspect of the current experiment was that there were no substantial treatments effects on plant community composition over the course of the experiment, given that the two most abundant grass species remained highly dominant. Although the soil mineralization cores
would not have addressed the direct contribution of plants to soil N availability (i.e. there were no living roots in the cores), changes in plant community composition can nevertheless have important indirect effects via changes to plant tissue quality and the distribution of roots in the soil.

2.6 References


Hu, Y. Y., Zhang, Z. W., Yang, G. J., Ding, C., & Lu, X. T. (2021)$^b$. Increases in substrate availability and decreases in soil pH drive the positive effects of nitrogen addition
on soil net nitrogen mineralization in a temperate meadow steppe. *Pedobiologia*, 89.


magnified by time and the number of global change factors imposed. *Proceedings of the National Academy of Sciences*, 116, 17867 - 17873.


Chapter 3

Short- versus long-term effects of nitrogen addition and warming on ecosystem nitrogen retention

3.1 Introduction

Global change drivers have the potential to disrupt nitrogen (N) cycling by altering ecosystem N retention (Li et al. 2021, Pornon et al. 2019, Yang et al. 2022), but their effects on N retention can shift substantially over time (Clark et al. 2009). Foremost, in response to increased atmospheric N deposition, ecosystem N losses can remain low in the short term if primary production remains N limited, with much of the additional reactive N incorporated into plant and microbial biomass (Menge 2011; Zhang et al. 2015). However, it has been well-documented that chronic inputs of N over the longer term can result in N saturation (Sheng et al. 2019, Vitousek et al. 1997), and after this threshold is crossed, additional inputs of N become increasingly vulnerable to losses through hydrological processes and trace gas emissions (Hoeft et al. 2014; Niu et al. 2016). Similarly, while climate warming can alter productivity by accelerating plant green-up in spring and extending the growing season, which can increase plant N retention (Guretzky et al. 2016, Reyes-Fox et al. 2014, Reyes-Fox et al. 2017) and increase soil microbial activity (Andresen et al. 2010, Birgander et al. 2018), microbial communities can develop increasingly efficient N and carbon recycling mechanisms in response to warming over the longer term (Bradford & Fierer 2012; Guo et al. 2018).

It has been suggested that over the short-term, the impacts of global change on N cycling and retention occur mainly directly through changes to soil N mineralization
(Contosta et al. 2011, Sun et al. 2013, Zhang et al. 2012), whereas over the longer term, changes to plant and soil community compositions become more influential (Gao & Yan, 2019; Guo et al. 2018). For example, Shi et al. (2018) observed a transition from an annual C3 dominated plant community to a perennial C4 dominated plant community in response to warming, and such shifts in plant functional types can have substantial effects on net N mineralization and N retention (Yao et al. 2011). Nevertheless, in global change field experiments, significant changes in plant species composition can take at least a decade to emerge, even in grassland systems (Komatsu et al. 2019; Luo et al. 2011).

Although data from many global change field experiments document only relatively short-term effects on ecosystem N cycling, data from an increasing number of longer-term experiments (e.g. > a decade) have emerged (Meeran et al. 2023, Savage et al. 2014, Wu et al. 2020). While the comparison of results from the early years of an experiment with those of the later years can reveal longer-term, cumulative effects on N processes, such comparisons can be confounded with interannual variation in the environment that could potentially interact with the experimental treatment effects (He et al. 2022; Zhou et al. 2022). For example, interannual variation in the timing and speed of snow melt in the spring can affect both the strength and direction of plant and soil microbial responses to experimental warming and N deposition, which ultimately affects N retention (Henry et al. 2015). Likewise, annual variation in precipitation and temperature can have large effects on related ecosystem metrics such as net ecosystem carbon storage (Novick 2004), and large effects of precipitation on ecosystem carbon and nitrogen assimilation have been documented in gradient studies (Feyissa et al. 2021). Therefore, in the context of a variable environment, changes in N retention over time may not represent cumulative ecosystem effects.
In this study, I compared the short-term versus long-term effects of N addition and warming on plant and soil N retention in a grass-dominated temperate old field using $^{15}$N stable isotope tracer added the in spring, prior to plant green-up. I then collected soil samples and shoots from the two dominant grass species, *Poa pratensis* and *Bromus inermis*, at the peak of the plant growing season, to assess $^{15}$N retention. A key element of the experimental design was that in addition to the original N addition and warming plots, which were 16 years old, a newer set of N addition, warming and control plots, interspersed among the original plots, was established for the last three years of the experiment. This design allowed the short- and long-term effects to be compared directly while controlling for possible variation in background environmental conditions. Based on the hypothesis that N limitation decreases with chronic N deposition, which increases N losses, I predicted the recovery of the added $^{15}$N tracer would be lower in old N addition plots than in the newer N addition plots. For warming, I predicted that the recovery of the added $^{15}$N tracer would be higher in the warmed plots due to the extended growing season and increased biomass production, and that differences in $^{15}$N retention between the new and old plots would be correlated with differences in biomass production.
3.2 Methods

3.2.1 Field site description

The field site, located in London, Ontario, Canada at the Agriculture Canada Southern Crop Protection and Food Research Centre (43°01´46´´N, 81°12´52´´W), was situated in a temperate old-field plant community. The land was previously used as an agricultural field 35 years ago and planted with rotations of wheat, corn, and soybean. Thereafter, it was seeded with grasses and left unmanaged. The two dominant species at the field site, which accounted for >93% of the aboveground plant biomass, were the introduced perennial cool-season grasses Poa pratensis L. and Bromus inermis Leyss. However, the forb species Cirsium arvense L., Asclepias syriaca L., Aster ericoides L., and Solidago altissima L., and the legume Lotus corniculatus L. were also present, although patchy in distribution. The soil has a composition of 9% clay, 41% silt, and 50% sand, an average pH of 7.6 (Bell et al. 2010), and it was classified as well to imperfectly drained silt loam glacial till (Hagerty & Kingston 1992). Mean annual air temperature from April 2022 – April 2023 was 9.3 °C, which was warmer than the regional average air temperature of 8.6 °C, and annual precipitation was 746 mm, which was drier than the average annual precipitation of 1055 mm (1981-2010 Climate Normal Data, Environment Canada, National Climate Data and Information Archive).

3.2.2 Warming and N addition treatments

The experimental was organized in a randomized block design (n=10), with two warming treatments (warming year-round and no warming) and two N addition treatments (nitrogen fertilization and ambient nitrogen). The longer-term plots were
established in late 2006 and consisted of 40 plots total: control, N addition only, warming only, and combined N addition and warming plots. The short-term plots, established in late 2019, consist of only 30 plots total, because they lack combined warming and N addition treatment (thus, the examination of plot age, warming and N addition was not completely factorial). The plots were circular (1.13 m in diameter) with a 10 cm buffer zone extending beyond the perimeter that also received the treatments. The warming treatment was administered with 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) suspended 50 cm above the soil in each plot. The ceramic infrared heaters warmed the plants and soil without giving off photosynthetically active radiation (Harte et al. 1995). The N addition treatments were administered each year and included a pulse of aqueous NH₄NO₃ added at a rate of 2 g m⁻² y⁻¹ in late March in combination with slow release NH₄NO₃ pellets (Florikan ESA, Sarasota, Fla.) added at a rate of 4 g m⁻² y⁻¹ in late May. The total rate of 6 g m⁻² y⁻¹ was selected based on high-end estimates for atmospheric N deposition projected for the region for 2050 (Galloway et al. 2004). Soil temperature was recorded hourly using 107-BAM temperature probes (Campbell Scientific Canada Corp., Edmonton, AB, Canada) located at 2 and 5 cm depth, while soil moisture was estimated hourly using CS616-L TDR probes (Campbell Scientific Canada Corp., Edmonton, AB, Canada) located from 0-15 cm and 0-30 cm depths. From May 2023 to July 2023, the datalogger files were corrupted, resulted in a loss of soil temperature and moisture data over this time.
- \( \bullet \) = IR heater
- \( \bigcirc \) = dummy heater
- 16 = old plot (16 years)
- 3 = new plot (3 years)
**Figure 3.1** Experimental plot design was the same as used in chapter 2, shown again here is a representative block (1 of 10). Plots consisted of short-term (<3 years) treatments, including control plots (C) and those receiving either N addition (N) or warming (W), and long-term (>16 years) treatments, including control plots (C) and those receiving either N addition (N), warming (W) or both N addition and warming combined (NW). Other plots, that were not used in this study, are denoted by an X.
Figure 3.2 The start (down arrows) and end (up arrows) indicate the timing of $^{15}$N stable isotope addition and the subsequent date for collection of sample material for $^{15}$N analysis, plotted against A) daily mean ambient air temperature, B) daily precipitation, and C) snow cover present at the field site from 1 January 2022 through 30 September 2022. In C), the black vertical lines indicate the presence of snow cover on the plots (C – ambient temperature plots; W – warmed plots).
3.2.3 $^{15}$N tracer addition and analyses

On 7 April 2022, following snowmelt, I added 0.1 g $^{15}$N m$^{-2}$ as $^{15}$NH$_4^{15}$NO$_3$ dissolved in water to a 30 cm diameter circular subplot within each of the 70 experimental plots. Prior to the addition of the $^{15}$N tracer, I also collected bulk soil and plant samples from outside the plots to determine the natural abundance of $^{15}$N in these N pools. Then, on 4 July 2022, which corresponded with the peak of the plant growing season, I collected the aboveground biomass of both Poa pratensis and Bromus inermis from a 10 cm $\times$ 10 cm square quadrat from the center of each subplot. I also collected a 2 cm diameter, 15 cm deep soil sample from each quadrat area. I dried both the soil and aboveground plant tissue at 60 °C for 3-4 days. The aboveground plant tissue was chopped into small pieces using a Wiley mill, then the milled plant material and soil were ground into powder using a ball mill (Spex Certiprep Geno/grinder 2000, Metuchen, NJ, USA). I weighed 3 mg of the ground plant tissue separately for each of the two grass species into 4 mm $\times$ 6 mm tin capsules, and 12 mg of the soil samples into 9 mm $\times$10 mm tin capsules. The samples were sent for total N and $^{15}$N analysis via mass spectroscopy at the Cornell University Stable Isotope Laboratory.

The $^{15}$N isotopic composition values supplied by the analytical laboratory were expressed in standard notation ($\delta^{15}$N) in parts per thousand relative to atmospheric N$_2$, where:

$$\delta^{15}N_{\text{sample}} = \left[ \frac{(^{15}N/^{14}N)_{\text{sample}}}{(^{15}N/^{14}N)_{\text{standard}}} - 1 \right] \times 1000$$

I used the $\delta^{15}$N values to determine the percent $^{15}$N by mass in the soil and plant samples. Then I subtracted the percent $^{15}$N in the natural abundance samples from the
total percent $^{15}$N in the soil and plant samples, to estimate excess $^{15}$N (i.e. the latter was attributed to the $^{15}$N tracer addition). I determined $^{15}$N retention per unit area by multiplying the concentration of excess $^{15}$N tracer found in each N pool by the total mass per area for the respective pools, then I compared these values to the amount of $^{15}$N added to each plot.

3.2.4 Statistical analyses

I conducted three two-way ANOVAs: 1) one examining the effects of N addition, plot age, and their interaction, 2) one examining the effects of warming, plot age, and their interaction, 3) and the final examining the effects of N addition, warming and their interaction in the old plots only, to analyze the treatment effects on total aboveground biomass of Poa pratensis, total aboveground biomass of Bromus inermis, % recovery of the $^{15}$N tracer in the Poa pratensis biomass, % recovery of the $^{15}$N tracer in the Bromus inermis biomass, and % recovery of the $^{15}$N tracer in the bulk soil. For each analysis, block was included in the model as a random factor.

3.3 Results

3.3.1 Recovery of the $^{15}$N label in Bromus inermis aboveground biomass

The mean recovery of added $^{15}$N in Bromus inermis aboveground biomass ranged from 4-10%. Bromus inermis aboveground biomass increased significantly in response to warming (P=0.013, Table 3.1), but did not respond significantly to N addition (Fig 3.3 A). Warming significantly increased the recovery of added $^{15}$N in Bromus inermis aboveground biomass (P=0.006), with more than twice as much of the label recovered in
plants in the warmed plots (Fig. 3.4 A, Table 3.1). However, there were no significant interactions between either of the treatments and plot age or between N addition and warming (the latter assessed for old plots only) for aboveground biomass or % $^{15}$N (Table 3.1).

3.3.2 Recovery of the $^{15}$N label in *Poa pratensis* aboveground biomass

The mean recovery of added $^{15}$N in *Poa pratensis* aboveground biomass ranged from 8-15%. *Poa pratensis* aboveground biomass increased significantly in response to N addition (P=0.034; Table 3.1), but it did not respond significantly to warming (Fig 3.3 A). There was also significantly higher percent tissue N in the *Poa pratensis* in the N addition plots (Table 3.2, P=0.003). There were no significant treatment effects on the % $^{15}$N recovery in aboveground biomass of *Poa pratensis* (Table 3.1). Similarly, there were no significant interactions between either of the treatments and plot age or between N addition and warming for aboveground biomass or recovery of the $^{15}$N label in *Poa pratensis* (Table 3.1).
**Figure 3.3** Mean aboveground biomass of A) *Bromus inermis* and B) *Poa pratensis* sampled on 4 July and 6 July 2022 in i) N addition plots and control plots (both old and new plots), ii) warmed plots and control plots (both old and new plots), and iii) N addition, warmed, combined, and control plots (old plots only). Error bars represent standard error (n=10) and significant ANOVA results are displayed on the panels. New plots refers to the 3-year-old treatment plots and old plots refers to the 16-year-old treatment plots. No N and N refer to the N control and N addition plots respectively, no W and W refer to the temperature control and warmed plots respectively, N + W refers to the combined treatment plots and no N,W refers to the old control plots.
**Figure 3.4** Mean % recovery of the $^{15}$N label in A) *Bromus inermis*, B) *Poa pratensis*, and C) bulk soil sampled on 4 July and 6 July 2022 in i) N addition plots and control plots (both old and new plots), ii) warmed plots and control plots (both old and new plots), and iii) N addition, warmed, combined, and control plots (old plots only). Error bars represent standard error (n=10) and significant ANOVA results are displayed on the panels. New plots refers to the 3-year-old treatment plots and old plots refers to the 16-year-old treatment plots. No N and N refer to the N control and N addition plots respectively, no W and W refer to the temperature control and warmed plots respectively, N + W refers to the combined treatment plots and no N,W refers to the old control plots.
Table 3.1 P values determined by three two-way ANOVAs testing for significant effects of 1) plot age, warming, and their interaction, 2) plot age, N addition, and their interaction, and 3) N addition, warming, and their interaction on biomass production of *Bromus inermis* and *Poa pratensis* and % recovery of the $^{15}$N isotope tracer in aboveground *Bromus inermis* biomass, aboveground *Poa pratensis* biomass, and bulk soil samples. Significant treatment effects are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Aboveground biomass</th>
<th>$^{15}$N % Recovery</th>
<th>% Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bromus inermis</em></td>
<td><em>Poa pratensis</em></td>
<td>Bulk Soil</td>
</tr>
<tr>
<td><strong>Old + New Plots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.292</td>
<td>0.145</td>
<td>0.909</td>
</tr>
<tr>
<td>N</td>
<td>0.429</td>
<td>0.411</td>
<td>0.528</td>
</tr>
<tr>
<td>N * Age</td>
<td>0.572</td>
<td>0.893</td>
<td>0.585</td>
</tr>
<tr>
<td><strong>Old + New Plots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.453</td>
<td>0.267</td>
<td>0.475</td>
</tr>
<tr>
<td>W</td>
<td><strong>0.013</strong></td>
<td>0.276</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>W * Age</td>
<td>0.285</td>
<td>0.531</td>
<td>0.189</td>
</tr>
<tr>
<td><strong>Old Plots Only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.905</td>
<td><strong>0.034</strong></td>
<td>0.606</td>
</tr>
<tr>
<td>W</td>
<td>0.236</td>
<td>0.321</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>N * W</td>
<td>0.923</td>
<td>0.175</td>
<td>0.675</td>
</tr>
</tbody>
</table>
Table 3.2 The percent nitrogen of *Bromus inermis*, *Poa pratensis*, and bulk soil samples. Standard errors for each mean are provided in parentheses.

<table>
<thead>
<tr>
<th>% Nitrogen</th>
<th>Treatments</th>
<th><em>Bromus inermis</em></th>
<th><em>Poa pratensis</em></th>
<th>Bulk Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old control</td>
<td>1.16 (0.16)</td>
<td>1.05 (0.13)</td>
<td>0.23 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Old N</td>
<td>1.43 (0.10)</td>
<td>1.40 (0.09)</td>
<td>0.25 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Old warming</td>
<td>1.41 (0.17)</td>
<td>1.24 (0.09)</td>
<td>0.24 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Old combined</td>
<td>1.30 (0.17)</td>
<td>1.45 (0.15)</td>
<td>0.26 (0.01)</td>
<td></td>
</tr>
<tr>
<td>New control</td>
<td>1.52 (0.14)</td>
<td>1.19 (0.07)</td>
<td>0.23 (0.01)</td>
<td></td>
</tr>
<tr>
<td>New N</td>
<td>1.36 (0.14)</td>
<td>1.46 (0.15)</td>
<td>0.24 (0.01)</td>
<td></td>
</tr>
<tr>
<td>New warming</td>
<td>1.17 (0.10)</td>
<td>1.16 (0.07)</td>
<td>0.23 (0.02)</td>
<td></td>
</tr>
</tbody>
</table>
3.3.3 Recovery of $^{15}\text{N}$ label in soil

The mean % recovery of $^{15}\text{N}$ label in soil was between 27-46%. Both warming (P=0.008; Fig 3.4 C) and N addition (P=0.016; Fig 3.4 C) significantly increased the recovery of $^{15}\text{N}$ label in the soil. However, despite a marginally significant interaction between N addition and plot age (P=0.09; Table 3.1) there were no strongly significant interactions between either of the treatments and plot age for soil $^{15}\text{N}$ retention.

3.4 Discussion

In short term field experiments, while the direct effects of global change treatments may have the largest effects on N cycling and N retention, over the longer term (e.g. decades), changes in plant and soil community composition may drive further changes (Gao & Yan, 2019; Komatsu et al. 2019). Field experiments that do not account for these changes in plant species composition may have poor predictive power for extrapolating the responses of ecosystem processes to the long term (Franklin et al. 2016; Zhou et al. 2020). Based on the potential for these long term cumulative effects, I predicted I would observe interactions between the N addition and warming treatments with plot age. However, contrary to my prediction, for $^{15}\text{N}$ retention there were no significant interactions between plot age for either N addition or warming. Nevertheless, the dominance of the two non-native grasses remained consistent over the course of the experiment, which was indicated by the lack of significant interactions between the treatments and plot age for their aboveground biomass. This result demonstrates how dominant plant species that resist replacement by other species may be able to buffer against the effects of global change (Compagnoni et al. 2021, Palit et al. 2021, Scarpitta et
In particular, although considered to be naturalized in some regions, both *Bromus inermis* and *Poa pratensis* are invasive grass species in North America (Dillemuth et al. 2009, Ellis-Felege et al. 2013, Grant et al. 2020, Spackman et al. 2020, White et al. 2013). In the case of old field plant communities dominated by such species, global change effects on the successional transition to dominance by woody species (Backhaus et al. 2021, Jochems et al. 2021, Shi et al. 2015) may play a key role in determining longer-term soil N responses (Habtewold et al. 2021, Jiang et al. 2021, Leptic et al. 2021).

Despite their similar functional roles as C3 grasses, *Bromus inermis* and *Poa pratensis* exhibit contrasting size and morphologies, which likely explains their different responses to the warming and N addition treatments. *Bromus inermis*, which grows much taller than *Poa pratensis*, can rapidly grow past the shade of the litter layer in spring to take advantage of the earlier growing season initiated by the heaters in the warmed plots (Dodd et al. 2005; Hutchison & Henry 2010), which would explain why *Bromus inermis*, and not *Poa pratensis*, primarily responded to the warming treatment, with respect to biomass production and $^{15}$N retention. This early season response of *Bromus inermis* highlights the key role of warming in promoting early season N retention by plants (Li et al. 2022; Turner & Henry 2009), at a time when N losses are otherwise high due to the combination of increased soluble N accumulation in soil over winter (Freppaz et al. 2012; Zhang et al. 2022), increased runoff and leaching caused by snow melt (Chapter 2, Jost et al 2011, Yano et al. 2010) and low plant N uptake caused by cold temperatures (Larsen et al. 2012). Thereafter, increased biomass production by grasses in response to warming can suppress the activities of other herbaceous plants through shading (Craig & Henry 2022, Deak et al. 2011, Wei et al. 2005), and grass litter produced in one year can further
suppress competing herbaceous plant species the next year, favouring rhizomatous grasses over other species (Hou et al. 2019, Zavaleta et al. 2003).

Unlike *Bromus inermis*, *Poa pratensis* forms a much denser mat of turf, with an extensive fibrous root system (Halvorson et al. 2020). The latter likely explains why *Poa pratensis* is highly responsive to increased addition of N fertilizer to the soil surface, allowing a much farther reach for mobile N in soils compared to other herbaceous plant species (Zhang et al. 2013), and why it primarily responded to N addition in our experiment. Notably, the bulk soil exhibited increased $^{15}$N retention in response to both warming and N addition, likely as a result of the respective increases in the *Bromus* and *Poa* root systems (documented in Craig & Henry 2022) and their associated soil microbial communities (Chen et al. 2021, Wardle et al. 2004, Yu et al. 2018). However, given the strong link between plant species composition and belowground N cycling processes (Aqeel et al. 2023, Kastovska et al. 2015, Schmidt et al. 2014), it was not surprising that the lack of interactions between plot age and the treatments for plant biomass corresponded with a lack of an interaction between plot age and the treatments for soil $^{15}$N retention.

Even in the absence of changes to plant species composition (Alkemade et al. 2011, Honu et al. 2006, Martin et al. 2019, Yang et al. 2011), chronic N deposition is anticipated to promote increased N leaching losses over time as a result of cumulative changes to soil N pools that result in N saturation (Niu et al. 2016, Uri et al. 2007, Zheng et al. 2023); high N retention in response to elevated N deposition has been documented over the short term (Ma et al. 2020, Perakis et al. 2005, Pornon et al. 2019), whereas decreased N retention is typically observed when N is applied for a greater period of time (Clark et al. 2009; Currie et al. 2002). In contrast, the lack of a strongly significant
interactive effect of plot age and N addition on $^{15}$N retention in my experiment indicated that the new N addition plots may already be reaching the point of N saturation after only three years, which was consistent with the leaching losses from both the old and new N addition being similar over the growing season (Chapter 2). This response may have resulted from the addition rate of 6 g N m$^{-2}$ y$^{-1}$ being on the high end of predicted future deposition rates for this region, coupled with the ambient deposition rate in the region already having reached 1-2 g N m$^{-2}$ y$^{-1}$. Similarly, Yang et al. (2023) observed that 6.70g N m$^{-2}$ y$^{-1}$ was sufficient to shift a temperate grassland into a state of N saturation in as little as 5 years. Both the old and new N addition plots in my experiment also exhibited similar percent soil N concentrations, which indicates that much of the N added to the old plots over the last decade has been lost from the system.

Despite the evidence for high absolute N losses from the N addition plots (i.e. based on the cumulative amount of N addition relative to the pools of remaining N), the N addition plots nevertheless exhibited greater soil $^{15}$N retention than the ambient N control plots. These results are consistent with the previous observation that N inputs can increase N retention by stimulating plant and microbial biomass production (Bai et al. 2021; Wang et al. 2022) and increasing the C:N ratio of biomass (Yang et al. 2022); increased soil $^{15}$N retention in the N addition plots in my experiment could be explained by increased belowground biomass (Craig & Henry 2022). My predictions at the outset of the experiment did not account for the potential for increased $^{15}$N retention in the N addition plots despite their overall increase in N losses - i.e. they retained a higher proportion of the added $^{15}$N, but they lost much more added N in an absolute sense ($^{15}$N-labelled and unlabelled N combined) because they were fertilized.
3.5 Conclusions

Overall, my results revealed that when controlling for variation in environmental conditions, there were no significant interactions between the N addition and warming treatments with plot age when comparing $^{15}$N retention between three year old and 16 year old plots. These results highlight the potentially important role of dominant species that resist replacement by other species in buffering against the effects of global change on cumulative changes in ecosystem N retention. Moving forward, these results also place an emphasis on determining how the global change factors may influence successional processes in this system, given they are the likely driver of future shifts in plant species composition.

3.6 References


Kermavnar, J., Widmer, S., Zimmermann, Z., Rofrics, N., de Bello, F., Leps, J., &
Medina, N. G. (2021). Shift from trait convergence to divergence along old-field

effects on root growth and soil aggregation: A meta-analysis. *Soil Biology &
Biochemistry*, 157.

Extracellular Enzyme activity and microbial biomass to warming and nitrogen

relationships to seasonal change and winter warming in a temperate grassland.

Bradford, M. A., & Fierer, N. (2012). The biogeography of microbial communities and
ecosystem processes: implications for soil and ecosystem models. *Soil Ecology
and Ecosystem Services*, 189 – 200.

Plant-mediated effects of long-term warming on soil microorganisms on the
Qinghai-Tibet Plateau. *Catena*, 204.

Clark, C. M., Hobbie, S. E., Venterea, R., & Tilman, D. (2009). Long-lasting effects on
nitrogen cycling 12 years after treatments cease despite minimal long-term


silver birch (Betula pendula Roth.) stand growing on abandoned agricultural land.


Chapter 4

General Discussion & Conclusions

4.1 Research findings

I examined the effects of N deposition and warming on ecosystem N dynamics over both the short-term (3 years) and the long-term (16 years). My initial predictions were correct for the treatment effects on belowground N cycling processes, such as net N mineralization and N leaching, and there was a significant interaction between plot age and N addition for total N mineralization over summer. However, aboveground plant responses to the treatment effects were delayed and contrary to my initial predictions there were no significant interactions between plot age and either of the treatments for $^{15}$N retention. Although there was significantly increased retention of the $^{15}$N tracer in bulk soil samples under both treatments, belowground N cycling dynamics are closely linked to aboveground plant communities (Wardle et al. 2004) and the absence of plant community processes to the treatments may further hinder the true response of ecosystem N dynamics under global change (Brumme & Jhanna 2008, Chen et al. 2015, Ma et al. 2011).

The first experiment captured mostly the responses of soil microbial community function and abiotic processes to global change (Auyeung et al. 2013, Bartczak et al. 2021, Zheng et al. 2017), which was contrary to the second experiment, which was heavily influenced by plant community responses to global change (Liu et al. 2017, McWhirter & Henry 2015, Zupan et al. 2017). It has been shown that a timescale of 3 years can capture shifts in microbial community compositions (Aqeel et al. 2023; Gao et
and increases to microbial activity (Liu et al. 2016; Zhou et al. 2013) that are characteristic of warming treatments. However, the timescale of the old, warmed plots (16 years) was not able to capture a shift in the plant community composition that is expected with longer-term warming treatments (Edwards & Henry, 2016, George et al. 2009, Shi et al. 2015), excluding a major ecosystem response to long-term global change (Avolio et al. 2019, Huang et al. 2021, Criado et al. 2020). This can be explained by the two dominant plant species at the field site being invasive grass species (Dillemuth et al. 2009; Ellis-Felege et al. 2013) that also have been shown to adapt well to environmental conditions that can suppress local vegetation (Fulbright et al. 2021; Langley et al. 2018, Langley et al. 2022) and disrupt colonization of the field by native species (Brown & Bugg, 2001, Dickson & Busby 2009, Grman et al. 2021, Smilauer & Smilauerova, 2013) through rhizomatous mats and high seedling densities (Craig & Henry 2022, Fisher et al. 1996, Kim et al. 2017, Kiss et al. 2022).

The first experiment was able to capture short- versus long-term treatment effects on belowground processes with a significant interaction between plot age and warming on total N mineralization over winter, as well as a significant interaction between plot age and N deposition over the growing season, since unlike plant community responses, soil microbial community responses happen much more rapidly (Zogg et al. 2000). Overall, these findings suggest that although individual processes such as increased retention of N under warming (Pornon et al. 2019, Wang et al. 2018, Xiao et al. 2022) and N addition (Bontti et al. 2011; Lu et al. 2011) may be realized under longer term global change experiments, in order to capture ecosystem N dynamics under global change over varying time scales, both aboveground (Gong et al. 2015, He et al. 2013, Komatsu et al. 2019, Monk & Gabrielson 1985) and belowground processes (Kaiser et al. 2011, Kastoyska et
al. 2015, Reay et al. 2023, Schmidt et al. 2014) must be present to capture the emerging responses of ecosystems.

### 4.2 Research limitations

When the experimental treatments were started 16 years ago, projections for atmospheric N deposition by the year 2050 in our region were between 2-5 g N m\(^{-2}\) y\(^{-1}\) (Galloway et al. 2004), which is why 6 g N m\(^{-2}\) y\(^{-1}\) was selected as the treatment level (i.e. just above the upper bound for projected N deposition). However, the current rate of background atmospheric N deposition received by the control plots is already 1-2 g N m\(^{-2}\) y\(^{-1}\). Moreover, there is evidence that in recent years, atmospheric N pollution has been decreasing due to more efficient burning of fossil fuels (Stamenic et al. 2019). Therefore, the rate of N addition in the experiment likely exceeds the total future N deposition over and above the current rate of deposition. This disparity may be meaningful, given that the average threshold for terrestrial ecosystems to switch from N limitation to N saturation appears to be 5 – 6g N m\(^{-2}\) y\(^{-2}\) (Tian et al. 2016; Yungfeng et al. 2020), and it would explain why N saturation may have been achieved after only years in the new N addition plots.

Regarding the soil mineralization and leaching measurements using soil cores, although this is a commonly used technique, it nevertheless has several well-documented shortcomings. Foremost, while it allows the vertical flow of water through the column and the exclusion of contamination from N outside the core, it also excludes live roots during the incubation period, and it includes severed roots, both of which could affect soil microbial activity. In addition this technique does not account for the role of live roots in
reducing N leaching. As for my study of $^{15}$N retention, my experiment only addressed the effects over the plant growing season. In a previous study in the initial years of the experiment, Turner & Henry (2009) showed that half of the $^{15}$N stored in plant shoots was lost over winter, although this $^{15}$N was recovered in the soil.

4.3 Directions for future research

While leaching of N from soil due to the shift from N limitation to N saturation is a major avenue for N loss, especially during periods of high water infiltration (e.g. snow melt), gaseous losses of N also can increase at these times. Specifically, anaerobic conditions that can occur in the soil over winter or during spring snow melt (Baumann et al. 1997), can accelerate denitrification and lead to substantial outputs of nitrous oxide ($\text{N}_2\text{O}$) and nitrogen gas ($\text{N}_2$) (Manucharoba et al. 1999). Such effects would be informative to examine in the experiment, given that nitrous oxide is a potent greenhouse gas (Vanamstel & Swart 1994).

A closer examination of belowground microbial processes also would be useful for further understanding the responses of soil N and carbon in the content of the experiment. For example, a recent study by Stroud et al. (2022), conducted on the experiment, demonstrated a cumulative effect of the warming treatment on enzyme activity and litter decomposition over time. Moreover, Stroud & Henry (2023) observed a 14% increase in in the particulate organic matter fraction in new N addition plots in response to warming and a 12% decrease in the particulate organic matter fraction in the old N addition plots. While the aboveground N cycling processes may be delayed in this experimental design, there appears to be rapid responses of belowground N cycling to the
treatments, and a detailed characterisation of microbial community composition could help explain these effects. Furthermore, to capture the entire ecosystem response to the experimental treatments, both aboveground plant and belowground microbial communities must be considered in tandem when determining the consequences of global change on N cycling.

4.4 References


Benjamin Souriol
Curriculum vitae

Degrees and Certificates

- Sept 2021-Current: MSc, Biology, University of Western Ontario. Major GPA Biology: 84%
- Sept 2012-June 2017: Honours BSc, Biology, minor in Chemistry; honours thesis in Chemistry, Nipissing University. Major GPA Biology: 74.5%; Chemistry: 78.2%; Last 20 courses: 82%; Thesis Research: 92%.
- Sept 2010-June 2011: Electrical Techniques Certificate, Canadore College

Awards

- 2016-2017: Carl Sanders Scholarship (based on grades in preceding academic year), Nipissing University
- 2023: Graduate Travel Award, University of Western Ontario
- 2023: OE3C 2023 Travel Award, University of Western Ontario

Presentations


Graduate Courses Taken and Graduate Teaching Assistantship

- 2022 Winter Graduate: Soil Ecology, 9442B, 81%
- 2023 Winter Graduate: Global Change Biology, 9419B, 87%
- 2021 Fall GTA: Ecology, 2483A
- 2022 Winter GTA: Patterns in the diversity of life, 3484B
- 2022 Fall GTA: Scientific methods in biology, 2290G
- 2023 Winter GTA: Patterns in the diversity of life 3484B

Research Experience

- Working with my Chemistry professor, Dr. Mukund Jha, I completed a year-long thesis project entitled “Organic chemistry synthesis of novel indole-fused thiopyrans and the exploration of intermolecular cyclization” earning 92%, in which I:
  - Synthesized and characterized novel indole-fused thiopyrans, expanding the library for this class of organic molecule
- Developed laboratory and instrumental techniques for devices such as nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), gas spectrometer and chromatography (TLC, HPLC, and column chromatography)
- Presented my thesis project at the end of year at a public poster presentation held at Nipissing University

- Working with my MSc supervisor, Dr. Hugh Henry, I am currently completing a 2 year-long thesis project entitled “Short-Term versus Long-Term effects of Nitrogen Addition and Warming on Ecosystem Nitrogen Dynamics in a Grass-Dominated Old Field”. In this research project I have:
  - Compared the short- vs. long-term effects of nitrogen addition and warming on soil nitrogen mineralization and leaching
  - Using in situ soil cores, I have quantified pools of inorganic nitrogen in both bulk soil and leaching pools, and developed laboratory and instrumental techniques for an elemental analyser to determine ammonium and nitrate concentrations in samples
  - Compared the short- vs. long-term effects of nitrogen addition and warming on plant and soil nitrogen retention
  - Using a stable isotope $^{15}$N tracer, I have compared the retention of the tracer in both plant and soil pools
  - By utilizing mass spectroscopy analysis, I have determined the $\%^{15}$N and $\%$N in the plant and soil samples
  - Presented my MSc entry talk, and my MSc exit talk, at the Ecology & Evolution Philosophical talks held at Western University